Journal of Medical Virology 61:181–186 (2000)

Prevalence of Drug Resistant Mutants and Virological Response to Combination Therapy in Patients With Primary HIV-1 Infection

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Baseline genotype resistance analysis was carried out in 48 adults with primary HIV-1 infection between 1995 and 1998 before starting early combination therapy. Seventeen percent (8/48) of the isolates displayed key mutations conferring resistance to reverse transcriptase (RT) inhibitors such as amino acid substitutions 215Y/F (5/48,10%), 70R (3/48, 6%), 184V (2%). Two percent (1/48) had a major mutation associated with resistance to protease inhibitors (D30N). Other mutations at positions 10, 15, 20, 33, 36, 46, 63, 71, 77, 82, 93 of the protease gene were frequent (73%). Among the 46 patients who were given antiretroviral combination therapy and who responded durably to treatment after 6 and 12 months, there was no significant difference between those harboring RT mutant strains (Group I) and those with wild-type isolates (Group II). No significant difference was found at months 6 and 12 between the two groups in terms of CD4+ cell counts. These findings suggest that the presence of drug-resistant strains at the time of primary HIV-1 infection does not necessarily predict drug failure. Other factors, such as adherence to treatment, tolerance and pharmacokinetics parameters are probably major determinants of virological response in patients with early therapeutic intervention. ***J. Med. Virol. 61: 181–186, 2000.***

KEY WORDS: primary HIV-1 infection; transmitted drug-resistant mutant; combination therapy

INTRODUCTION

Routine analysis of HIV-1 reverse transcriptase (RT) and protease genes has given insight on the prevalence of key mutations that confer resistance to various antiretroviral drugs [Hirsch et al., 1998; Larder et al., 1999]. Recently, it has been shown that sequence analysis of HIV-1 reverse transcriptase (RT) and protease genes could give valuable information for choosing or adapting therapeutic regimen [Durant et al., 1999]. In this respect, primary HIV infection is a clinical situation that still needs to be evaluated. Theoretically, the increasing number of available antiretroviral drugs and the larger access to combination therapy can lead to the transmission of resistant viruses [Erice et al., 1993; Imrie et al., 1997; Hecht et al., 1998]. One important issue, that remains to be resolved, is the actual influence of transmitted resistance mutations at the time of primary HIV infection on the virologic response to antiretroviral treatment [Hirsch et al., 1998]. Viruses transmitted with major resistance mutations could indeed result in partial virologic response or even therapy failure.

The aim of this study was to evaluate the prevalence of transmitted drug-resistant mutants in 48 patients with primary HIV infection and to assess whether the presence of such mutations affects virological response.

MATERIALS AND METHODS

Patients

Forty-eight subjects (28 men and 20 women) attending the Marseille and Toulouse AIDS centers were included in the study between 1995 and 1998. Enrollment criterion included recent exposure to infection within the last 3 months confirmed by clinical or laboratory findings. Laboratory criteria confirming acute

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Accepted 19 November 1999

HIV were: a negative serology by third generation ELISA with a positive p24 antigenemia or plasma HIV-1 RNA load, or a typical seroconversion pattern; all patients but four were seen within the first month after the clinical symptoms of primary HIV infection. Transmission routes were homosexual (21 men) or heterosexual (27 men or women) intercourse. The most frequent clinical symptoms of primary HIV infection consisted of acute retroviral syndrome (42 of the 48 subjects) with fever, pharyngitis, lymphadenopathy.

Forty-six patients included in the study provided written informed consent before given antiretroviral combination that was started between 10–21 days after diagnosis. Peripheral blood samples were collected before starting therapy then at 1- and 3-month intervals by venipuncture in syringes containing EDTA, transported at ambient temperature to the laboratory within 4 hr and stored at –80°C until testing for HIV RNA concentration that was measured before initiating treatment and genotyping. In this study, sequence analysis was not used to choose an optimal antiretroviral regimen.

Antiretroviral regimens were as follows: double therapy in 5 cases, zidovudine + didanosine, 2 patients; stavudine + didanosine or lamivudine, 3 patients. Triple antiretroviral treatment with nucleoside inhibitors including a combination of zidovudine (250 mg, twice daily), didanosine (200 mg, twice daily), zalcitabine (0.75 mg, 3 times daily) or lamivudine (150 mg, twice daily) (12 patients); triple therapy with nucleoside inhibitors plus a protease inhibitor (29 patients), indinavir (18 patients), saquinavir (1 patient), ritonavir (8 patients), nelfinavir (2 patients). All patients were given treatment for a minimal period of 12 months. Compliance to treatment was assessed by monthly interview.

Virological Monitoring

Plasma HIV-1 RNA was measured using the Amplicor HIV-1 Monitor RT-PCR assay (Roche Diagnostic Systems, Neuilly, France) according to the manufacturer's instructions. Plasma samples containing no detectable RNA by the standard procedures were tested using the ultrasensitive procedure (cut-off <20 copies/ml) of Amplicor HIV-1 Monitor TM version 1.5. [Schockmel et al., 1997].

Genotype Analysis of HIV-1 RT and Protease Genes

HIV RNA from plasma samples was assayed as described previously [Tamalet et al., 1998]. Briefly, HIV RNA was purified from 1 ml of plasma using the QIAamp viral RNA kit (Qiagen, Courtaboeuf, France). cDNA synthesis was carried out with superscript I RnaseH$^-$- reverse transcriptase (Gibco BRL). The cDNA was amplified by nested PCR with Taq DNA polymerase and supplied buffer (Boehringer, Mannheim, Germany). For RT, an 800 base-pair fragment was sequenced after amplification with primers RT19/RT20 (first round), MJ3/MJ4 (second round) and A20/NE120 (sequencing). The entire protease gene was amplified using the following pairs of primers: 5′epb/3′epb (first round) and 5′pb/3′pb (second round and sequencing). The purified PCR products were sequenced using the ABI PRISM Dye Terminator cycle sequencing kit with AmpliTaq Polymerase FS (Applied Biosystems, Foster City, CA). Sequence products were purified and analyzed using the Applied Biosystems 377 automatic sequencing system. The sequences were aligned with HIV-1 strain HXB2 RT and protease genes, using sequence Navigator Software (Applied Biosystems).

The primer sequences were as follows: RT19, GCACATAAAGCTATAGGTACAG; RT20, CTGCCAGTTCTAGCTCTGCTTC; MJ3, AGTAGGACCTACACCTGTCAAC; MJ4, CTGTTAGTGCTTTGGTTCCTCT; A20, ATTTTCCCATTAGTCCTATT; NE120, ATGTCATTGACAGTCCAGCT; 3′epb, TTTTGGGCCATCCATTCCTGGCTT; 3′pb, ACTGGTACAGTTTCAAATAGG; 5′epb, AGAGCTTCAGGTCTGGGG; 5′pb, GAAGCAGGAGCCGATAGACA.

As recommended recently by the International AIDS Society-USA panel, "primary" or major mutations conferring drug resistance by themselves were distinguished from "secondary" or accessory mutations that could improve the fitness of virus containing primary mutations [Hirsch et al., 1998].

CD4 Lymphocyte Counts

Peripheral blood CD4-lymphocytes were counted by flow cytometry (Epics Profile; Coulter, Hialeah, FL) using commercially available monoclonal antibodies (Beckton-Dickinson, Mountain View, CA).

Statistical Analysis

HIV-1 RNA concentrations were transformed to $\log_{10}$ values before analysis. Specimens in which HIV RNA was undetectable were assigned the value of the detection limit of the ultrasensitive monitor assay, i.e., 1.3 log copies/ml. Quantitative variables were analyzed by a Student's *t*-test. A *P* value of less than 0.05 was considered significant.

RESULTS

Patient Population

Forty-eight patients entered this retrospective study. In the first plasma sample, plasma HIV-1 RNA was positive in each patient. HIV-1 western blot was negative or indeterminate in 14 cases, displayed a seroreactivity compatible with a seroconversion pattern in 30 cases, and full reactivity in 4 cases. Twelve patients were treated within 10 days after the first clinical symptoms or evidence of virological diagnosis of primary HIV-1 infection and 34 between 2 and 3 weeks. One treated patient was lost to follow-up after 6 months.

At baseline, the mean CD4 T cell count was 564 ± 231/mm^3. Mean plasma HIV-1 RNA level was 5.35 ± 1.09 log copies/ml; in 66% cases, patient viral load was

TABLE I. Aminoacid Substitutions in HIV-1 Reverse Transcriptase and Protease Genes From Subjects With PHI During the 1995–1998 Period

Year	No. of patients with PHI	Patients harbouring strains with major mutations	
		Reverse transcriptase gene	Protease gene
1995	12	3/12[a,b,c]	0/12
1996	14	3/14[d,e,f]	0/14
1997	18	2/18[g,h]	1/18[h]
1998	4	0/4	0/4
Total	48	8/48	1/48

PHI, primary HIV infection.
[a]K70R.
[b]D67N, T215F, K219E.
[c]K70R, K219Q.
[d]K70R.
[e]M41L, T215Y.
[f]M41L, D67N, T69D, L210W, T215Y.
[g]T215Y.
[h]M41L, M184V, T215Y (RT gene) and D30N, L63P, V77I, I93L (Protease gene).

>5 log; in 23% it was between 4 and 5 log; whereas in 11%, it was <4 log.

Detection of Drug Resistance Mutations

Genotypic analysis displayed the presence of major mutations associated with resistance to reverse transcriptase inhibitors in 8 of the 48 patients (17%) as summarized in Table I. The mutations were detected as pure mutant populations. These changes included the major 215F/Y amino-acid substitution (5 cases, 10%), and K70R (3/48, 6%) that are related to the resistance to zidovudine; although the K70R mutation can represent just a polymorphism [Najera et al., 1995; Dietrich et al., 1997]. The M184V amino acid substitution that is involved in the resistance to lamivudine was detected in 1 patient (2%) and associated with the mutation at codon 215. Other amino acid substitutions associated with major mutations in the RT gene were detected at positions 41, 67, 69, 210 and 219. In addition, in 1 case, a T215S substitution was observed. Protease major mutation was detected in only 1 of 48 patients (2%) harboring the D30N amino acid substitution associated with resistance to nelfinavir [Markowitz et al., 1998]. Minor mutations or natural polymorphism in the protease gene were observed in 35/48 patients (73%). The frequency of substitutions at different positions of protease gene was as follows: L63P/A (32 cases,67%); V77T/I/L (11 cases, 23%); I93L (6 cases, 1.3%); A71V/T (4 cases, 8%); M36I (3 cases, 6%); L10I (2 cases, 4%). Finally, substitutions at positions I15V, K20R, L33V, M46I, V82I were each seen in one case (2%). The description of resistance mutations per year during the 1995–97 period is shown in Table I.

The influence of NRTI mutations on the virological response was assessed in the 46 treated patients. A division into two groups of patients was made on the presence or absence of mutations at baseline (Table II). Seven patients infected with drug-resistant viruses (Group I) had viruses harboring AZT major resistance mutations; in 1 case, in addition to AZT resistance mutations, a M184V mutation associated with 3TC resistance was also detected. Thirty-nine patients were infected with wild-type viruses (Group II). Patients of both groups received triple therapy with or without protease inhibitors, except 5 patients in Group II who were given a combination of 2 nucleoside RT-inhibitors. All patients but 3 in Group II received AZT as part of their therapy regimen.

Response to antiretroviral therapy was assessed by CD4+ cell count and quantification of HIV-1 plasma viral load at months 6 and 12. The proportion of patients from Groups I and II who achieved complete virologic suppression, as manifested by plasma HIV-1 RNA load below the limit quantification of the assay, i.e., 20 copies/ml, was 3/7 and 15/39 respectively at month 6, 2/7 and 20/38 respectively at month 12 (data not shown). As shown in Table II, no significant difference was found in terms of mean change of HIV-1 RNA expressed as $\Delta\log_{10}$ HIV-1 RNA copies/ml, and CD4+ cell count from the baseline up to months 6 and 12, between the 2 groups.

A major protease inhibitor resistance- associated mutation was observed in only 1 patient who harbored a strain with a D30N substitution conferring resistance to nelfinavir plus a T215Y/M41L/M184V mutated pattern of reverse transcriptase; a phenotypic test using a conventional culture method [Brun-Vezinet et al., 1992] was carried out on the cell-associated virus isolated at baseline that demonstrated partial resistance to zidovudine (IC_{50} = 0.36 μM) and high level resistance to lamivudine (IC_{50} > 6.25 μM). This patient experienced a complete durable virological response to a regimen including zidovudine + lamivudine + indinavir as shown in Figure 1.

DISCUSSION

The present study describes the resistance variants characterized at the time of acute HIV infection in Southern France and reports the virological response to combination therapy according to the presence or absence of preexisting drug resistance-associated mutations.

The proportion of NRTI resistance mutations (T215Y, 10%; M184V, 2%) and of major protease mutations (D30N, 2%) was close to most recent European, Australian and US reports [Harzic et al., 1999; Miller et al., 1999; Little et al., 1999]. Strains containing mutations associated with non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance, as previously reported [Imrie et al., 1997; Hecht et al., 1998] were not found. Despite an increasing number of patients treated between 1995 and 1998, an increase in transmitted resistance mutations was not observed (Table I). A French study in antiretroviral drug-naive patients infected for a median period of 5.3 years found a lower prevalence rate (1.2%) of transmitted resistance mutations (T215Y, 1%) [Descamps et al., 1999]. In this last study, however, a small increase in the number of

TABLE II. Influence of NRTI Resistance-Associated Mutations on the Virological and Immunological Responses*

	Δ $\log_{10}$ HIV-1 RNA copies/ml		Δ CD4+ cell count (/mm^3)	
	M6	M12	M6	M12
Patients on treatment infected by viruses with major NRTI resistance mutations (Group I) n = 7	−2.3 ± 1.9	−2.3 ± 1.7	+186 ± 264	+271 ± 236
Patients on treatment infected by wild-type viruses (Group II) n = 39	−3.0 ± 1.9	−2.8 ± 2.2	+110 ± 280	+100 ± 265
All patients n = 46	−2.8 ± 2.0	−2.7 ± 2.2	+107 ± 283	+115 ± 275

*NRTI, Nucleoside reverse transcriptase inhibitors. Group I vs. Group II: not significant for all comparisons. Plasma viral load decreases were expressed as $\log_{10}$ HIV-1 RNA copies/ml differences between measures at baseline and after 6 months (M6) and 12 months (M12) of treatment.

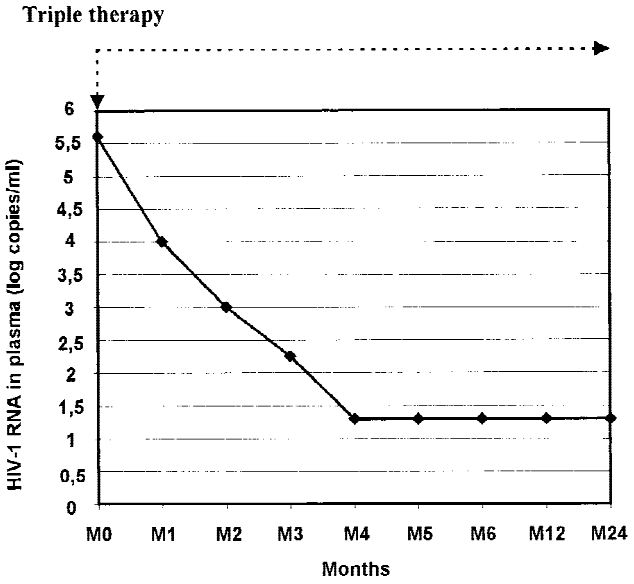

Fig. 1. Changes in RNA concentration in plasma for Patient h infected by a multidrug resistant virus and treated with zidovudine + lamivudine + indinavir.

transmitted resistance mutations and multiple resistance was observed between 1996 and 1998.

In the present study, a T215S substitution was observed in one patient enrolled at a late stage of seroconversion (western blot fully reactive) and could reflect in the absence of drug pressure for a short time period the replacement of the viral population with the resistance-conferring tyrosine (TAC) codon 215 by a more fit variant with a single mutation at codon 215 creating serine (TCC) as described previously [Goudsmit et al., 1996]. Recently, De Ronde et al. [1999] reported that in a drug-naive newly infected subject, the absence of drug selection pressure resulted in the replacement of an AZT-resistant population with T215Y by a variant D/S with an increase in fitness [De Ronde et al., 1999].

The low frequency of transmitted strains resistant to protease inhibitors did not allow the evaluation of the impact of major protease resistance mutations on viral responses. Indeed, only one strain harbored a major protease substitution (D30N). Fortunately, this patient was not given nelfinavir but did receive indinavir, a drug with a non-overlapping drug resistance profile. Moreover, as recently pointed out [Bossi et al., 1999], natural polymorphism of protease gene does not have any influence on the response to treatment including a protease inhibitor (data not shown).

Development of drug resistance is considered as one of the major causes of treatment failure [Lorenzi et al., 1999], but the clinical relevance of baseline mutations is still not well defined. Patients a, b, c and d receiving a triple therapy including zidovudine + didanosine + lamivudine were infected with strains harboring zidovudine -resistance associated mutations (a: K70R; b: T215F, D67N, K219E; c: K70R, K219Q; d: K70R) and were fully or partially responders at month 12 of therapy. The codon 70 mutation, however, could also reflect a natural polymorphism as noted previously [Najera et al., 1995; Dietrich et al., 1997]. Moreover, mutated genotypes are not necessarily correlated with a resistance phenotype as reported by Weinstock who observed a decrease in susceptibility to zidovudine in only 1 out of the 6 patients with mutations associated with zidovudine resistance [Weinstock et al., 1999]. In addition, in some cases, resistance mutations associated with a complete or partial phenotypic resistance do not necessarily affect virological response to highly active antiretroviral therapy as observed in Patient h in whom the virus carried major mutations in the RT gene (M41L, M184V, T215Y) and a major mutation in the protease gene (D30N). Interestingly, the zidovudine + lamivudine + indinavir combination given to this patient was fully efficient over a follow-up of 24 months despite a high level resistance of the baseline strain to lamivudine and a partial resistance to zidovudine (data not shown). This observation also confirms that the D30N substitution does not impair the response to indinavir, as pointed out recently by Condra et al. [1999] in the context of nelfinavir-experienced patients. The potency of indinavir against a homogeneous viral strain at the time of primary HIV-1 infection [Ho, 1995] could explain the sustained virological effect in this patient. In addition, the impaired replicative capacity of polymutated drug-resistant variants [Goudsmit et al., 1996] could contribute to the viral suppression associated with an efficient host immune response controlling the viremia [Borrow et al., 1994; Rosenberg et al., 1997] as expressed in particular by a relatively high rate of CD4+ T cells (716 at month 24). This observation suggests that outcome is not affected

systematically by baseline resistance mutations in newly-infected individuals starting combination therapy, although such RT and protease mutations may strongly predispose individuals to fail highly active antiretroviral therapy [Harrigan et al., 1999].

These data extend a recent short-term study by Rubio et al. [1998] who demonstrated the lack of significant differences in CD4+ cell count and HIV-1 RNA 24-week responses to zidovudine plus didanosine/zalcitabine therapy between drug-naive patients with or without ZDV resistance at baseline. Clinical questioning revealed that one of the major determinants of therapeutic efficacy in responders as well as in nonresponders, was adherence to treatment [Altice and Friedland, 1998]. In the group of nonresponders, however, one cannot rule out a possible lack of pharmacological effect of drugs due to a defect in intestinal absorption or poor metabolism at an individual level also contributing to the reduction of potency of antiretrovirals [Rodriguez-Rosado et al., 1998].

These data, although limited to a small number of patients, suggest that despite resistant variants, the viral population at the time of primary HIV-1 infection is homogeneous enough to be more susceptible to antiretrovirals than in multi-experienced patients. Now that the question of hitting the virus "early and hard" is under debate due to the toxic effects and impact on quality of life [Hirschel and Francioli, 1998; Carr et al., 1998], these results indicate that highly active antiretroviral therapy [Miller et al., 1999] at the time of primary HIV-1 infection is useful for overcoming risks of insufficient virological response due to transmitted resistance to antiviral drugs.

The determination of RT and protease genotypes at the earliest time after viral transmission remains of first importance in the context of epidemiologic survey and as a baseline characterization of the patient's strain. As the question of knowing whether baseline-resistance mutations at primary HIV-1 infection affect or not the outcome is still an important issue under debate, further studies are needed to evaluate the impact of genotypic determinations for the choice of the initial antiretroviral regimen.

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